JOURNAL OF BACTERIOLOGY, June 2004, p. 3674–3676 Vol. 186, No. 12
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.12.3674–3676.2004

Reciprocal Regulation of Bioluminescence and Type III Protein Secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus* in Response to Diffusible Chemical Signals

Stephen C. Winans*

Department of Microbiology, Cornell University, Ithaca, New York

It has long been known that some groups of bacteria exhibit complex patterns of coordinated behavior and that in several cases, the behaviors of these populations of cells are regulated by diffusible chemical signals. These behaviors include bioluminescence, the horizontal transfer of DNA, the formation of biofilms, and the production of pathogenesis factors, antibiotics, and other secondary metabolites (32). A broad variety of signal molecules and signal receptors have been identified over the past 20 years. Of these, possibly the most intriguing signal consists of a molecule called autoinducer 2 (AI-2), which is made by a wide variety of bacteria. The discovery of AI-2, by Bonnie Bassler and her colleagues at Princeton University, sent shock waves through the scientific community, since their discoveries suggested, first, that virtually all bacteria might send and receive chemical signals, and second, that these bacteria might signal intergenerically, using what has been termed a "bacterial Esperanto." What did all these bacteria need to say to each other? AI-2 was first discovered in the marine bioluminescent bacterium *Vibrio harveyi*, which uses two parallel signaling pathways to control expression of its *lux* operon. In this issue, the Bassler group reports that this communication system also regulates expression of a type III secretion (TTS) system, which presumably translocates specific proteins to eukaryotic host cells (10a). Surprisingly, expression of the TTS system and the *lux* operon are inversely regulated in that AI-1 and AI-2 repress the former but activate that latter.

Most known bacterial signaling systems fall into two classes. Gram-positive bacteria typically communicate using oligopeptide signals that are detected by two-component phosphorelay pathways (8), while proteobacteria generally signal via acylhomoserine lactones (AHLs) that are synthesized by proteins that resemble LuxI of *Vibrio fischeri* and are detected by transcription factors that resemble LuxR *of V. fischeri*. These systems are sometimes referred to as quorum-sensing regulators, which suggests that the purpose of these systems is to estimate population density. It is unfortunate that this term is used so freely, since there is little or no evidence that the purpose of any of these signaling systems is to take a bacterial census. It is just as likely that these systems are designed to coordinate the behavior of cell populations rather than to enumerate them.

The signaling system of *V. harveyi* does not resemble either of the family systems described above. Instead, *V. harveyi* signals using AI-2 and another chemical signal called AI-1 (1, 2). AI-1 is 3-hydroxy-butanoylhomoserine lactone, which is a member of the family of AHL signals (3). Surprisingly, the AI-1 synthase (LuxM) does not resemble members of the LuxI family, although it is a member of a second small family of synthases that so far has been found only in the genus *Vibrio*. Detection of AI-1 requires a membrane-spanning two-component kinase (LuxN), while detection of AI-2 requires a periplasmic protein that resembles the ribose binding protein (LuxP) (2) and a second two-component kinase (LuxQ) (1, 2). Both LuxN and LuxQ are hybrid proteins containing a sensor kinase domain and a response regulator domain that funnel phosphoryl groups via LuxU to LuxO, a σ^{54}-dependent transcriptional activator that is hypothesized to control the expression of an unidentified repressor of *luxR*. LuxR (not related to the *V. fischeri* LuxR protein) activates the luciferase structural operon (*luxCDABE*) (2, 9, 16). In the absence of both signals, the LuxN and LuxQ proteins act as kinases and the resulting phosporyl-LuxO blocks luminescence. In the presence of signals, the proteins act as phosphatases and the ultimate dephosphorylation of LuxO stimulates luminescence. Both signals are needed for bioluminescence, because in the absence of one signal the cognate receptor acts as a potent kinase to block *lux* gene expression (20).

For a number of years, the gene directing synthesis of AI-2 remained undiscovered. In 1999, the *luxS* gene was identified in *V. harveyi* and a very similar gene was described in *Salmonella enterica* serovar Typhimurium (29). Since that time *luxS* genes have been found in dozens of bacterial genera, and the structures of four LuxS proteins have been determined by X-ray crystallography (11, 15, 21). LuxS catalyzes a step in the turnover of *S*-adenosyl methionine (SAM) (22, 23). When SAM is used as a methyl donor, *S*-adenosyl-homocysteine (SAH) is generated as one of the products. The adenyl group of SAH is removed by the Pfs protein, generating *S*-ribosylhomocysteine (SRH). The LuxS protein hydrolyzes SRH, forming homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), which cyclizes spontaneously to form active AI-2. The precise chemical structure of AI-2 was solved by X-ray crystallography of the ligand-bound form of the AI-2 receptor (LuxP) (4). As predicted, AI-2 consists in part of a cyclized carbohydrate that vaguely resembles ribose, from which it is derived. Unexpectedly, the LuxP structure showed a molecule of borate covalently bonded to the carbohydrate, indicating that AI-2 is a furanosyl borate diester. Boron is an abundant component of seawater, and so it should be available to the bacteria. It was a complete surprise that AI-2 would include borate, and the significance of this finding is unclear.

Perhaps the most striking finding about AI-2 is that it is synthesized by so many bacterial genera. The *luxS* gene is

* Mailing address: Department of Microbiology, 361A Wing Hall, Cornell University, Ithaca, NY 14853. Phone: (607) 255-2413. Fax: (607) 255-3904. E-mail: scw2@cornell.edu.

highly conserved in most groups of bacteria, although it is absent from the alpha proteobacteria (19, 29). Many of the bacteria that encode a *luxS* gene have been shown to produce AI-2, and in every case tested, disruption of *luxS* eliminated AI-2 production (6, 12, 14, 17, 26, 31). AI-2 appears to have diverse roles in signaling. Pathogenic *Escherichia coli* strains increase the expression of a Type III protein translocation system in response to AI-2 (24), and in transcriptional profiling experiments, large numbers of *E. coli* genes are either up-regulated or down-regulated by this signal (7, 26). AI-2 also controls hemin acquisition genes in *Porphyromonas gingivalis* (27), the expression of the VirB virulence factor in *Shigella flexneri* (5), and the secretion of the SpeB cysteine protease virulence determinant of *Streptococcus pyogenes* (6). The detection of AI-2 by these bacteria is largely uncharacterized, although work from the Bassler lab indicates that *S. enterica* serovar Typhimurium detects AI-2 by the use of an ABC-type uptake system coupled to a transcriptional repressor that is inactivated by phospho-AI-2. This is fundamentally different from AI-2 detection by *V. harveyi* (29, 30). Perhaps the most important unanswered question about AI-2 is whether all the bacteria that make it actually use it in signaling, as AI-2 may be released by some bacteria as a waste product rather than a signal.

In the current study from the Bassler laboratory, genes that are regulated by the *V. harveyi* signaling system were sought by testing a library of random *lac* fusions for increased or decreased β-galactosidase expression in the presence of these signals. Eight target genes were identified, including one that encodes a σ^{54}-dependent response regulator and another that encodes a hybrid two-component kinase–response regulator. It is interesting that these genes were identified, as the AI-1 and AI-2 transduction circuitry includes both classes of proteins. Another regulated gene identified in the study encodes a methyl-accepting chemoreceptor, suggesting that these signals might influence chemotaxis. These findings indicate that this signaling system regulates diverse functions and lies within a signal transduction cascade.

The current study focuses on another group of genes that are regulated by AI-1 and AI-2. These genes encode a TTS system. TTS systems are found in a wide variety of bacterial pathogens (as well as the plant symbiotic bacteria *Rhizobium* spp.) and mediate the translocation of so-called effector proteins from bacteria directly into the host cell cytosol. These effector proteins act in a variety of ways to enhance the survival of the bacteria and their colonization of the host. Sequence analysis indicates that all TTS systems share a common ancestry and that they all evolved from the basal body of the bacterial flagellum (10). The genes that encode TTS systems are frequently clustered on pathogenicity islands that are known or suspected to engage in horizontal transfer. In the current study from the Bassler laboratory, the TTS system of *V. harveyi* was shown to be functional by demonstration that a protein called VopD, which resembles YopD of *Yersinia enterocolitica*, is released from the cells in strains containing an intact secretion system but not from those in mutant strains.

V. harveyi was not previously known to encode a TTS system. However, the closely related human pathogen *V. parahaemolyticus* was predicted by genomic sequencing to have such a system. The authors showed that the *V. parahaemolyticus* system was functional by showing that it too could secrete VopD. Furthermore, the *V. parahaemolyticus* system was shown to be regulated by the orthologous AI-1 and AI-2 signaling system found in this organism.

This is not the first report of a TTS system being regulated by diffusible signals, since the corresponding systems of enteropathogenic *E. coli* and enterohemorrhagic *E. coli* are regulated by AI-2 (26–28). In *E. coli*, however, AI-2 stimulates expression of these genes whereas in the Bassler study, AI-2 (and AI-1) inhibited expression. In another study of an enterohemorrhagic *E. coli* strain, the SdiA protein (homologous to the LuxR protein of *V. fischeri*) was observed to inhibit expression of the genes required for pedestal formation (13). Evidence for a signaling molecule was provided, although the signal was not identified. While SdiA appears able to detect a variety of AHL signals (18, 25), *E. coli* is not known to synthesize any AHLs (18). Thus, the natural signal detected by SdiA remains unknown.

It is not difficult to imagine how *V. harveyi* could regulate *lux* genes and TTS genes in a reciprocal manner. It is highly plausible, for example, that LuxR could activate the former genes but repress the latter. This could be accomplished simply by the positioning of the LuxR binding site with respect to the two promoters. The interesting question is not how but why these genes are regulated reciprocally. One might naively think that secretion of effector proteins would occur only at population densities sufficiently high for signaling. Here the AI-1 and AI-2 signals provide information to stop protein translocation. That is to say, it appears that solitary cells inject proteins into host cells whereas populations of cells do not. It must follow that in these *Vibrio* species, protein injection must occur at the very outset of host colonization before significant bacterial cell division.

I thank C. White for critical reading of this manuscript and the editors of the Journal of Bacteriology for the invitation to submit this commentary.

Research in my laboratory is supported by grants from the National Institutes of Health (GM42893) and from the National Science Foundation (MCB-9904917). The views expressed in this commentary do not necessarily reflect the views of the journal or of ASM.

REFERENCES

1. **Bassler, B. L., M. Wright, R. E. Showalter, and M. R. Silverman.** 1993. Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. Mol. Microbiol. **9:**773–786.
2. **Bassler, B. L., M. Wright, and M. R. Silverman.** 1994. Sequence and function of LuxO, a negative regulator of luminescence in *Vibrio harveyi*. Mol. Microbiol. **12:**403–412.
3. **Cao, J. G., and E. A. Meighen.** 1989. Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*. J. Biol. Chem. **264:**21670–21676.
4. **Chen, X., S. Schauder, N. Potier, A. Van Dorsselaer, I. Pelczer, B. L. Bassler, and F. M. Hughson.** 2002. Structural identification of a bacterial quorum-sensing signal containing boron. Nature **415:**545–549.
5. **Chung, W. O., Y. Park, R. J. Lamont, R. McNab, B. Barbieri, and D. R. Demuth.** 2001. Signaling system in *Porphyromonas gingivalis* based on a LuxS protein. J. Bacteriol. **183:**3903–3909.
6. **Day, W. A., Jr., and A. T. Maurelli.** 2001. *Shigella flexneri* LuxS quorum-sensing system modulates *virB* expression but is not essential for virulence. Infect. Immun. **69:**15–23.
7. **DeLisa, M. P., C. F. Wu, L. Wang, J. J. Valdes, and W. E. Bentley.** 2001. DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in *Escherichia coli*. J. Bacteriol. **183:**5239–5247.
8. **Dunny, G. M., and B. A. Leonard.** 1997. Cell-cell communication in gram-positive bacteria. Annu. Rev. Microbiol. **51:**527–564.
9. **Freeman, J. A., and B. L. Bassler.** 1999. Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. J. Bacteriol. **181:**899–906.

10. **Galan, J. E., and A. Collmer.** 1999. Type III secretion machines: bacterial devices for protein delivery into host cells. Science **284:**1322–1328.
10a. **Henke, J. M., and B. L. Bassler.** 2004. Quorum sensing regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. J. Bacteriol. **186:** 1322–1325.
11. **Hilgers, M. T., and M. L. Ludwig.** 2001. Crystal structure of the quorum-sensing protein LuxS reveals a catalytic metal site. Proc. Natl. Acad. Sci. USA **98:**11169–11174.
12. **Joyce, E. A., B. L. Bassler, and A. Wright.** 2000. Evidence for a signaling system in *Helicobacter pylori*: detection of a *luxS*-encoded autoinducer. J. Bacteriol. **182:**3638–3643.
13. **Kanamaru, K., I. Tatsuno, T. Tobe, and C. Sasakawa.** 2000. SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. **38:**805–816.
14. **Kim, S. Y., S. E. Lee, Y. R. Kim, C. M. Kim, P. Y. Ryu, H. E. Choy, S. S. Chung, and J. H. Rhee.** 2003. Regulation of *Vibrio vulnificus* virulence by the LuxS quorum-sensing system. Mol. Microbiol. **48:**1647–1664.
15. **Lewis, H. A., E. B. Furlong, B. Laubert, G. A. Eroshkina, Y. Batiyenko, J. M. Adams, M. G. Bergseid, C. D. Marsh, T. S. Peat, W. E. Sanderson, J. M. Sauder, and S. G. Buchanan.** 2001. A structural genomics approach to the study of quorum sensing: crystal structures of three LuxS orthologs. Structure (Cambridge) **9:**527–537.
16. **Lilley, B. N., and B. L. Bassler.** 2000. Regulation of quorum sensing in *Vibrio harveyi* by LuxO and sigma-54. Mol. Microbiol. **36:**940–954.
17. **Lyon, W. R., J. C. Madden, J. C. Levin, J. L. Stein, and M. G. Caparon.** 2001. Mutation of *luxS* affects growth and virulence factor expression in *Streptococcus pyogenes*. Mol. Microbiol. **42:**145–157.
18. **Michael, B., J. N. Smith, S. Swift, F. Heffron, and B. M. Ahmer.** 2001. SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. J. Bacteriol. **183:**5733–5742.
19. **Miller, M. B., and B. L. Bassler.** 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. **55:**165–199.
20. **Mok, K. C., N. S. Wingreen, and B. L. Bassler.** 2003. *Vibrio harveyi* quorum sensing: a coincidence detector for two autoinducers controls gene expression. EMBO J. **22:**870–881.
21. **Ruzheinikov, S. N., S. K. Das, S. E. Sedelnikova, A. Hartley, S. J. Foster, M. J. Horsburgh, A. G. Cox, C. W. McCleod, A. Mekhalfia, G. M. Blackburn, D. W. Rice, and P. J. Baker.** 2001. The 1.2 A structure of a novel quorum-sensing protein, *Bacillus subtilis* LuxS. J. Mol. Biol. **313:**111–122.
22. **Schauder, S., and B. L. Bassler.** 2001. The languages of bacteria. Genes Dev. **15:**1468–1480.
23. **Schauder, S., K. Shokat, M. G. Surette, and B. L. Bassler.** 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol. Microbiol. **41:**463–476.
24. **Shimizu, S., S. Shiozaki, T. Ohshiro, and H. Yamada.** 1984. Occurrence of S-adenosylhomocysteine hydrolase in prokaryote cells. Characterization of the enzyme from *Alcaligenes faecalis* and role of the enzyme in the activated methyl cycle. Eur. J. Biochem. **141:**385–392.
25. **Smith, J. N., and B. M. Ahmer.** 2003. Detection of other microbial species by *Salmonella*: expression of the SdiA regulon. J. Bacteriol. **185:**1357–1366.
26. **Sperandio, V., J. L. Mellies, W. Nguyen, S. Shin, and J. B. Kaper.** 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA **96:**15196–15201.
27. **Sperandio, V., A. G. Torres, J. A. Giron, and J. B. Kaper.** 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. J. Bacteriol. **183:**5187–5197.
28. **Sperandio, V., A. G. Torres, B. Jarvis, J. P. Nataro, and J. B. Kaper.** 2003. Bacteria-host communication: the language of hormones. Proc. Natl. Acad. Sci. USA **100:**8951–8956.
29. **Surette, M. G., M. B. Miller, and B. L. Bassler.** 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA **96:**1639–1644.
30. **Taga, M. E., S. T. Miller, and B. L. Bassler.** 2003. Lsr-mediated transport and processing of AI-2 in *Salmonella typhimurium*. Mol. Microbiol. **50:**1411–1427.
31. **Taga, M. E., J. L. Semmelhack, and B. L. Bassler.** 2001. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol. Microbiol. **42:**777–793.
32. **Whitehead, N. A., A. M. Barnard, H. Slater, N. J. Simpson, and G. P. Salmond.** 2001. Quorum-sensing in Gram-negative bacteria. FEMS Microbiol. Rev. **25:**365–404.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.